The Changes of Some Physiological Parameters in Prussian Carp under the Action of the Tilt Fungicide

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Abstract. Lately, the chemical means of plant protection are expanding so fast that they are overgrowing the research possibilities that want not only to prove their efficiency, but also the way in which the integrity of natural environment is ensured.

In this paper we study the action of such a product – the Tilt fungicide (the active substance is the propiconazole), under different concentration (0.265, 0.53, 1.06, 2.12 and 4.14 mg/l water) on some physiological parameters (oxygen consumption, breathing frequency, glycaemia and the number of erythrocytes) on prussian carp (Carassius auratus gibelio Bloch). In all the researched concentrations, the propiconazole modified the values of physiological parameters.

Keywords: propiconazole, fungicide, Prussian carp

INTRODUCTION

Propiconazole was first developed in 1979 by Janssen Pharmaceuticals of Belgium. The chemical denomination of propiconazole is: 1-[[2(2,4-Dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]1-H-1,2,4-triazole.

The active ingredient propiconazole is a triazole fungicide that has protective, curative, and systemic activity. Some trade names for products containing propiconazole include Tilt, Banner, Benit, Desmel, Orbit, Radar, Fidis, Alamo, Spire, Fractis, Bumper, Mantis, Restore, Banner Maxx, Taspa, Juno, Novel and Break. Propiconazole is a systemic foliar fungicide with a broad range of activity. It is used on grasses grown for seed, mushrooms, corn, wild rice, peanuts, almonds, sorghum, oats, pecans, apricots, peaches, nectarines, plums and prunes (Worthing, 1983).

Propiconazole's mode of action is demethylation of C-14 during ergosterol biosynthesis, and leading to accumulation of C-14 methyl sterols. The biosynthesis of these ergosterols is critical to the formation of cell walls of fungi (Cotrău et all, 1992, Dindea and Igna, 1986). This lack of normal sterol production slows or stops the growth of the fungus, effectively preventing further infection and/or invasion of host tissues. Therefore, propiconazole is considered to be fungistatic or growth inhibiting rather than fungicidal or killing.

The propiconazole is non-toxic for man, bees, invertebrates and soil bacterias, but is dangerous for fish and other aquatic organisms. Propiconazole had the following EC50/LC50 values in ppm for freshwater fish species: bluegill 1.3-10.2, brown trout 3.3, rainbow trout 0.9-13.2, carp 6.8-21.0, catfish 2.0-5.1, and fathead minnow 7.6.
MATERIALS AND METHODS

Determinations were made between January and April 2007 on Prussian carp samples (Carassius auratus gibelio Bloch), captured from the surrounding lakes of Pitești. After 10 days of adaptation in the lab, when they were fed ad libitum once a day, the fish were separated in lots, which were used separately for the following experiments:

The first experiment was carried out with Prussian carps individuals separated in five lots, each lot being subdivided in two sublots (ten fish): (1) individuals younger than one year, with an average weight of 12.91 g (C₀) and (2) individuals with an average weight of 38.08 g (C₁). The concentrations of propiconazole used in the first experiment were those of 0.265, 0.53, 1.06, 2.12 and respectively 4.14 mg/l water.

The second experiment was carried out with ten Prussian carp individuals having an average weight of 37.11 g, which were subjected to propiconazole concentrations of 1.06, and respectively 2.12 mg/l water. In order to determine the number of erythrocytes and glycaemia, three different lots of Prussian carp were used.

During all the experimental methods, the propiconazole came from the commercial product Tilt 250. The Tilt concentrations that have been used have been established by preliminary survival test. The immersion of fish in these solutions has been made after they have been well stirred and aired for five minutes. The water temperature has been between 18 and 20°C and the immersion solution has been changed every 48 hours and the water has been continuously aired; the fish have no been fed during the experiments, in order to avoid the intervention of this factor (Năstăsescu, 1986, Picoș and Năstăsescu, 1988).

The energetic metabolism, expressed by the oxygen consumption, was determined by using the closed respiratory chamber method (the oxygen dose in the water was established by using the Winkler chemical method) (Năstăsescu, 1986, Picoș and Năstăsescu, 1988). These determinations were made at intervals of 6, 24, 48, 72, 96, 168 and respectively 336 hours. However, in some cases the determinations were made at intervals shorter than 24 hours from the immersion.

The breathing frequency was determined at the same intervals as in the case of the energetic metabolism. The number of erythrocytes was microscopically determined with a Thoma cells numbering chamber, by using a small amount of blood collected from the caudal artery (Motelică et all, 1965, Picoș and Năstăsescu, 1988); the glycaemia has been determine using a glucometer. The measurements were carried out only for the 7 days treatments with Prussian carp individuals subjected to a concentration of 1.06 mg of propiconazole per liter of water.

The dates were statistically interpreted using the Anova test.

RESULTS AND DISCUSSION

In all experimental variants that have been applied on fish, all the stages described by Schäperclaus in the symptomatological scheme for the intoxicated fish (Năstăsescu, 1986, Picoș and Năstăsescu, 1988), have only been observed in the variant with the propiconazole in concentration of 4.14 mg/l water, where they have succeeded each other at very short intervals (the fish died in the first 24 experimental hours). For the other variants we have only noticed the first three stages.

For a better comparasion between the toxic effects of propiconazole in the studied concentrations, the average values of oxygen consumption have been graphicly represented in figure 1. Except the variant with propiconazole in concentration of 1.06mg/l water, for the
small fish overall, the fungicide had an inhibiting effect on oxygen consumption for the prussian carp (after shorter or longer periods of time, it has also been noticed a growth of the energetic metabolism, comparing with the values recorded 24 hours ago), the values recorded at the end of experiments, being, in all cases, significantly difference comparing to the control values (for p<0.05). The decrease of oxygen consumption upon the action of some pesticides (Dithane M-45, 30 and 50 mg/l) was observed by Marinescu et all (2004) and Mălăcea (1969).

In all studied concentration (0.265, 0.53, 1.06, 2.12 și 4.14 mg /l water), the propiconazole modified the values of breathing frequency as shown in figure 2. For the first two concentrations, the fungicide effect is stimulating at first and then inhibiting of the breathing frequency (in some cases between these two effect, a longer or shorter stationary period of the breathing frequency comes up); for the middle concentrations, the propiconazole effect is strongly inhibiting first, tending to reestablish the values of this physiological parameter (tendance that has not been kept till the end of the interval for the concentration of 1.106 mg/l water, or until the end of the acute test for the concentration of 2.12 mg/l water); at the concentration of 4.14mg/l, the propiconazole effect of breathing frequency has been strongly inhibiting. The values recorded at the end of the experiments, were, in all cases, significantly difference comparing to the control values (for p<0.05).

![Fig. 1. The influence of the propiconazole fungicide upon oxygen consumption on prussian carp](image_url)

- 0.265mg/l, 22.96 g
- 0.53 mg/l, 27.78 g
- 1.06 mg/l, 20.45 g
- 2.12 mg/l, 31.4 g
- 4.14 mg/l, 11.7 g
The glycaemia level in the fish individuals subjected for seven and four days to immersion into water with 1.06 and 2.12 mg/l of propiconazole, was also significantly affected as shown in figure 3. After seven days of immersion the Prussian carp to the fungicide action in concentration of 1.06 mg/l, the glycaemia values are higher than the ones of the controls (with 6.35% comparing to the control value). But, after an only four day’s exposal (the acute test) at propiconazole with the concentration of 2.12 mg/l, we notice a decrease of this metabolic parameter (with 7.1% comparing to the control value). A possible explanation for the propiconazole’s effect on glycaemia can be its toxic action on liver that represents the haemostatic center of the entire organism.
The number of erythrocytes in the fish individuals subjected for seven and four days to immersion into water with 1.06 and 2.12 mg/l of propiconazole, was also significantly affected (figure 4). The difference between the number of erythrocytes which was determined for the control (1386455 per ml) and the ‘treated’ lot (2057628, respectively 1943380 per ml), an average increase of 48.4% and 40.16% was found in the treated fish individuals, which seemed to be related to the acute decrease in the oxygen consumption.

Fig. 4. The influence of propiconazole fungicide upon number of erythrocytes on prussian carp

CONCLUSIONS

The propiconazole (that came from the commercial product „Tilt”), under the concentrations of 0.265, 0.53, 2.12 and 4.14 mg/l water, had, overall, an inhibitory effect on oxygen consumption for the prussian carp (Carassius auratus gibelio Bloch).

In all the researched concentrations (0.265, 0.53, 1.06, 2.12 and 4.14 mg/l water), the propiconazole modified the values of breathing frequency for the prussian carp, the values recorded at the end of the experiments were, in all cases, significantly different comparing to the control values (for p<0.05). For the first two concentrations, the fungicide’s effect is stimulating at first, then inhibiting for the frequency of the breathing movements, and for the middle concentrations the effect is strongly inhibiting in the first stage.

In the concentration of 1.06 mg/l, the propiconazole produces, after seven days of immersion, an increase of the glycaemia values, until at a 2.12 mg/l concentration, after an exposal of four days of the fish, there has been noticed a decrease of the values of this physiological parameter.

The number of erythrocytes has significantly increased after seven and four days of immersion (6) at propiconazole concentrations of 1.06 and 2.12 mg/l.

REFERENCES


